PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference 12567320 | FOR FURTHER ACTION | See Form PCT/IPEA/416 |
|---|---|---|
| International application No. PCT/AU2005/000168 | International filing date *(day/month/year)* 11 February 2005 | Priority date *(day/month/year)* 12 February 2004 |

International Patent Classification (IPC) or national classification and IPC

Int. Cl.

*C12N 15/12* (2006.01) *C12N 15/63* (2006.01) *C12N 15/90* (2006.01)

Applicant

THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH et al

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. [X] *(sent to the applicant and to the International Bureau)* a total of 4 sheets, as follows:
      - [ ] sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      - [ ] sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.

   b. [ ] *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or table related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - [X] Box No. I Basis of the report
   - [ ] Box No. II Priority
   - [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - [ ] Box No. IV Lack of unity of invention
   - [X] Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - [ ] Box No. VI Certain documents cited
   - [ ] Box No. VII Certain defects in the international application
   - [ ] Box No. VIII Certain observations on the international application

| Date of submission of the demand 17 November 2005 | Date of completion of this report 06 June 2006 |
|---|---|
| Name and mailing address of the IPEA/AU<br>AUSTRALIAN PATENT OFFICE<br>PO BOX 200, WODEN ACT 2606, AUSTRALIA<br>E-mail address: pct@ipaustralia.gov.au<br>Facsimile No. (02) 6285 3929 | Authorized Officer<br>**Sophina Calanni**<br>Telephone No. (02) 6283 2038 |

Form PCT/IPEA/409 (Cover sheet) (April 2005)

**Box No. I Basis of the report**

1. With regard to the language, this report is based on:

[X] The international application in the language in which it was filed

[ ] A translation of the international application into , which is the language of a translation furnished for the purposes of:

[ ] international search (under Rules 12.3(a) and 23.1 (b))

[ ] publication of the international application (under Rule 12.4(a))

[ ] international preliminary examination (Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

[ ] the international application as originally filed/furnished

[X] the description:

pages **1-83** as originally filed/furnished
pages* received by this Authority on with the letter of
pages* received by this Authority on with the letter of

[X] the claims:

pages as originally filed/furnished
pages* as amended (together with any statement) under Article 19
pages* **84-89** received by this Authority on **12 May 2006** with the letter of 12 May 2006.
pages* received by this Authority on with the letter of

[X] the drawings:

pages **1/42-42/42** as originally filed/furnished
pages* received by this Authority on with the letter of
pages* received by this Authority on with the letter of

[X] a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages
[ ] the claims, Nos.
[ ] the drawings, sheets/figs
[ ] the sequence listing *(specify)*:
[ ] any table(s) related to the sequence listing *(specify)*:

4. [ ] This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

[ ] the description, pages
[ ] the claims, Nos.
[ ] the drawings, sheets/figs
[ ] the sequence listing *(specify)*:
[ ] any table(s) related to the sequence listing *(specify)*:

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

| | | |
|---|---|---|
| Novelty (N) | Claims 1-47 | YES |
| | Claims | NO |
| Inventive step (IS) | Claims 1-47 | YES |
| | Claims | NO |
| Industrial applicability (IA) | Claims 1-47 | YES |
| | Claims | NO |

2. Citations and explanations (Rule 70.7)

The present application relates to model system to identify haematopoietic cells of particular lineages and their stage of differentiation. In particular, the specification discloses the use of a gene targeting strategy where an eGFP expression cassette is inserted into an intron of the Blimp-1 genomic allele. The strategy disclosed makes use of a targeting construct that comprises genomic sequences adjacent to a Blimp-1 exon, a splice acceptor site, internal ribosome entry site (IRES), eGFP cDNA and polyadenylation signal. Following homologous recombination eGFP is expressed from bicistronic mRNA under the control of the endogenous Blimp-1 regulatory elements. As such, eGFP expression within cells modified in this manner reflects the expression of Blimp-1. Blimp-1 expression has been linked to the terminal differentiation of B cells and other hematopoietic cells, thus monitoring the expression of GFP using this system permits the determination of the stage of hematopoietic differentiation.

The following documents identified in the International Search Report have been considered for the purposes of this report:

**D1** Knödel, M. et al., 1999, Reversal of blimp-1 mediated apoptosis by A1, a member of the Bcl-2 family, *European Journal of Immunology*, 29: 2988-2998.

**D2** Baxendale, S. et al., 2004, The B-cell maturation factor Blimp-1 specifies vertebrate slow-twitch muscle fiber identity in response to Hedgehog signalling, *Nature Genetics*, 36(1): 88-93.

**D3** Tunyaplin, C. et al., 2000, Characterisation of the B lymphocyte-induced maturation protein-1 (Blimp-1) gene, mRNA isoforms and basal promoter, *Nucleic Acids Research*, 28(24): 4846-4855.

**D4** Reljic, R. et al., 2000, Suppression of signal transducer and activator of transcription 3-dependent B lymphocyte terminal differentiation by BCL-6, *Journal of Experimental Medicine*, 192(12): 1841-1847.

Continued in Supplemental Box

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claims 1-47 are not fully supported by the disclosure provided in the specification.

In the present specification the coexpression of *Blimp-1* and a reporter molecule (e.g. eGFP) under the control of endogenous *Blimp* regulatory elements is achieved by modifying the *Blimp-1* allele such that it comprises an IRES and cDNA encoding a reporter molecule. The inclusion of these sequences enables the transcription of a bicistronic construct that expresses *Blimp-1* and the reporter molecule under the control of endogenous *Blimp* regulatory elements.

The present claims are not limited to the use of the specific strategies (as described above) that achieve bicistronic expression of reporter molecule under the control of the endogenous Blimp1 regulatory elements. Therefore the claims are not fully supported by the specification.

Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material
      - [X] a sequence listing
      - [ ] table(s) related to the sequence listing

   b. format of material
      - [X] on paper
      - [X] in electronic form

   c. time of filing/furnishing
      - [X] contained in the international application as filed
      - [X] filed together with the international application in electronic form
      - [ ] furnished subsequently to this Authority for the purposes of search and/or examination
      - [ ] received by this Authority as an amendment* on

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

Please note that although the present application contains a sequence listing the sequences contained therein were not searched.

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

Supplemental Box

Continuation of: Box V

Novelty (N) and Inventive Step (IS)

D1-D4 disclose various expression systems where Blimp-1(or a non-functional portion thereof) is coexpressed with a reporter molecule. The Blimp-1 polypeptide is not expressed under the control of endogenous Blimp-1 regulatory elements, rather the expression systems disclosed utilise exogenous regulatory elements that have been cloned into a vector and introduced into the host cell. As such, the citations do not disclose the present invention , therefore the subject matter of claims 1-47 is new and meets the requirements of Article 33(2) PCT with regard to novelty.

In addition, claims 1-47 meet the criteria set out in PCT Article 33(3) with regard to the requirement of Inventive Step because the prior art does not obviously suggest to a person skilled in the art the modification of cells such that they are capable of coexpressing Blimp-1 and a reporter molecule under the control of endogenous Blimp-1 regulatory elements, wherein the presence of Blimp is associated with a cellular phenotype or a commitment in the cell to terminally differentiate